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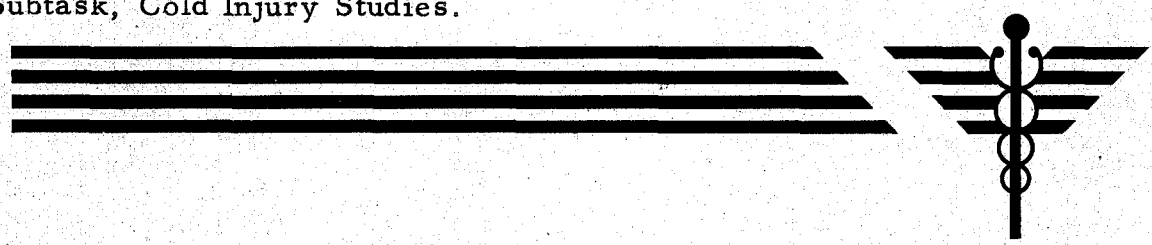
ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 81
14 April 1952

SICKLE CELL TRAIT AND FROSTBITE

*Subtask under Environmental Physiology, AMRL Project No. 6-64-12-028 Subtask, Cold Injury Studies.



MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

REPORT NO. 81

SICKLE CELL TRAIT AND FROSTBITE

by

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ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
14 April 1952

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ABSTRACT

SICKLE CELL TRAIT AND FROSTBITE

OBJECT

To determine the importance of the sickle cell trait as a predisposing factor in the pathogenesis of cold injury.

RESULTS

The sickle cell trait occurred with essentially equal frequency in a group of 346 control Negroes (10.4%) and a frostbite group of 66 cases (13.6%).

The sickle trait occurred with essentially equal frequency in a group of control cases tested before exposure to cold (10.9%) and a similar group tested after prolonged exposure to cold (9.6%).

There was no significant difference between the sickling and non-sickling distribution in the four main blood groups when the frostbite and control groups were compared.

The four main blood groups appeared with equal frequency in the frostbite and control groups.

No significant correlation was found between the occurrence of the sickle cell trait and the presence of cold agglutinins in the frostbite, pre-exposure control and post-exposure control groups.

CONCLUSIONS

The presence of the sickle cell trait in Negroes does not appear to be a significant factor in the causation of frostbite in this group.

The appearance of the sickle cell trait apparently is not influenced by exposure to cold.

Sickling has no apparent relationship to blood group agglutinogens in Negroes.

Sickling has no apparent relationship to cold agglutinins in control subjects and in frostbite cases.

RECOMMENDATIONS

None.

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SICKLE CELL TRAIT AND FROSTBITE

I. INTRODUCTION

Approximately 7 per cent of normal Negroes exhibit the sickle cell trait (2, 5, 6, 11), a Mendelian dominant inherited tendency, which is easily demonstrated by exposing the red blood cells to a diminished oxygen environment or to the *in vitro* action of a number of reducing agents (13). In perhaps 1 of 40 Negroes with this trait, sufficient *in vivo* destruction of red cells will result in a clinical entity, sickle cell anemia (2, 5, 10). A particularly important cause of symptoms in this disease is the occurrence of multiple vascular thrombotic occlusions, a resultant of the intravascular clumping of these peculiar elongated cells (3). Little is known of the relationship of the sickle cell trait to the presence of cold agglutinins or the blood group agglutinogens. One author found no correlation between the tendency to sickling and the presence of agglutinogens of the ABMN blood groups (1), and two investigations have shown that cold agglutinins are found in sickle cell anemia (8, 14).

Among the factors postulated in the pathogenesis of frostbite are certain phenomena associated with slowing of the capillary blood flow: intravascular agglutination, clumping of red cells, exudation of serum, and possibly "sludging" of blood. Recent evidence has accrued indicating the possibility that the presence of cold hemagglutinins may accelerate more complete red cell agglutination when a vascular area is exposed to low temperatures (12).

Recent military operations in cold weather have shown that Negro troops are more susceptible to cold injury. Careless use of clothing and neglect of proper protection are probably the most important determinants in the pathogenesis of cold injury in these troops. Since intravascular agglutination may be an important factor in the evolution of cold injury, and since Negro troops have high cold hemagglutinin titers (12), as well as the sickle cell trait, an attempt was made to study the role of sickling in the development of cold injury, its relation to cold agglutinins, and its relation to the main blood group agglutinogens.

II. EXPERIMENTAL

All studies were done at Camp Drum, New York during Exercise Snow Fall, from 6 January to 23 February 1952. Sickling tests were performed only on Negro subjects. Such troops were derived mainly from the following commands: 1) 11th Airborne Division; 2) 278th Regimental Combat Team; 3) 306th Logistical Command; 4) 3rd Armored Cavalry Regiment. Actual maneuvers occurred from 9 February to 16 February, 1952.

A. Methods

1. Subjects.

a. Pre-maneuver (Pre-exposure group).

Sickle cell tests were performed on 221 randomly selected Negro troops before maneuvers were begun. A total of 221 sickle cell preparations were studied (one for each subject).

b. Frostbite group.

Sickling preparations were performed on each of 66 Negro troops who manifested clinical frostbite during the maneuver time.

c. Post-maneuver (post-exposure group).

A total of 125 clinically normal subjects were tested for the sickle cell trait in the week following the maneuver.

2. Procedures

a. Sickle cell preparations.

One drop of venous blood was placed on a glass slide. The area was surrounded by petroleum jelly and covered by a glass cover slip, in effect sealing the red cells from any contact with the air. After a 24 hour period, during which all slides were kept at room temperature, the slide was read under high power and oil immersion magnification. By this method, sickling was evident, when present, in almost all fields examined. All slides were read by one of us (M. J. E.).

b. Cold agglutinins

All tests were performed as in a previous study by Weiner (12). Titers were read by one of us (M. J. E.).

c. Blood groups.

All data on blood groups were obtained by direct questioning of troops confirmed by examination of identification tags. Blood typing was not done as a rule.

d. Statistical analyses were performed by the chi square method with reference to tables of P values as an index of probability of significance (4, 7, 15).

B. Results

1. Sickle cell tests were performed on 412 subjects, according to the groupings mentioned above. The sickle cell trait was present in 10.9 per cent of the pre-exposure controls, 9.6 per cent of the post-exposure controls and 13.6 per cent of the frostbite cases. Since statistical analysis of pre- and post-exposure groups showed no significant difference in sickling incidence ($p = 0.71$), these two groups were considered as homogeneous for purposes of comparison with the frostbite group. Therefore, a control group of 346 cases showed an incidence of 10.4 per cent sickling. Comparison with the frostbite group showed no significant difference ($p = 0.90$). Table I summarizes the results obtained.

TABLE I

SICKLING IN FROSTBITE AND CONTROL SUBJECTS

GROUPS	CASES	SICKLING	%	p
Pre-exposure	221	24	10.9	0.71
Post-exposure	125	12	9.6	
Pre and Post exposure	346	36	10.4	0.90
Frostbite	66	9	13.6	
TOTAL	412	45	10.9	

2. Both sickle cell tests and blood group data were obtained on 40 cases with frostbite and in 332 control subjects. The control group was further divided into 209 pre-maneuver and 123 post-maneuver subjects. There was no significant difference in blood group frequency between these latter groups. This was to be expected since blood groups are inherited traits. Table II. summarizes the division by sickle cell trait of the frostbite subjects into the four main blood groups.

TABLE II
SICKLING AND BLOOD GROUPS IN FROSTBITE SUBJECTS

Blood Groups	Sickle Cell Positive	Sickle Cell Negative	Combined Data
O	5 (20.0%)	20 (80.0%)	25 (100.0%)
A	3 (33.3%)	6 (66.7%)	9 (100.0%)
B	1 (25.0%)	3 (75.0%)	4 (100.0%)
AB	0 (0.0%)	2 (100.0%)	2 (100.0%)

The presence or absence of sickling in both the frostbite subjects (Table II) and the control subjects (Table III) was found to have no statistically significant relation to their blood grouping*. Table III summarizes the incidence of sickling within the four blood groups in the pre-maneuver and post-maneuver control subjects.

TABLE III
SICKLING AND BLOOD GROUPS IN CONTROL SUBJECTS

	Blood Groups	Sickle Cell Positive	Sickle Cell Negative	Combined Data
Pre-exposure	O	15 (13.4%)	97 (86.6%)	112 (100.0%)
	A	2 (3.8%)	50 (96.2%)	52 (100.0%)
	B	6 (15.8%)	32 (84.2%)	38 (100.0%)
	AB	0 (0.0%)	7 (100.0%)	7 (100.0%)
	Total	23	186	209
Post-exposure	O	10 (12.0%)	73 (88.0%)	83 (100.0%)
	A	1 (4.2%)	23 (95.8%)	24 (100.0%)
	B	1 (7.7%)	12 (92.3%)	13 (100.0%)
	AB	0 (0.0%)	3 (100.0%)	3 (100.0%)
	Total	12	111	123

* Frostbite Subjects p = 0.18
Pre-exposure Subjects p = 0.15
Post-Exposure Subjects p = 0.19

Table IV summarizes the blood group frequency in the frostbite and control groups. No significant difference is apparent between the two groups.

TABLE IV
BLOOD GROUPS IN FROSTBITE AND CONTROL SUBJECTS

BLOOD GROUP	CONTROL	FROSTBITE
O	195 (58.7%)	25 (62.5%)
A	76 (22.9%)	9 (22.5%)
B	51 (15.4%)	4 (10.0%)
AB	3 (3.0%)	2 (5.0%)

3. Both sickle cell tests and cold agglutinin titers were obtained on 49 frostbite and 332 control subjects. The control subjects have been divided into a pre-exposure group (209 cases) and a post-exposure group (123 cases). Table V summarizes the results of cold agglutinin studies in frostbite subjects according to presence of sickle cell trait.

TABLE V
SICKLE CELL TESTS AND COLD AGGLUTININ TITERS IN FROSTBITE SUBJECTS

		SICKLE CELL NEGATIVE	SICKLE CELL POSITIVE	COMBINED
Total		41	8	49
Positive Aggl.		30 (73.1%)	5 (62.5%)	35 (73.5%)
Negative Aggl.		11 (26.9%)	3 (37.5%)	14 (26.5%)
TITERS	0	11	3	14
	1:2	17	5	22
	1:4	9		9
	1:8	2		2
	1:16			
	1:32			
	1:64	1		1
	1:128	1		1

Within the group of frostbite subjects analyzed the presence of the sickle cell trait was not associated with a significantly different amount of positive cold agglutinins. The number of subjects available for analysis was small.

Table VI summarizes the results of 209 pre-exposure cases. Positive titers of cold agglutinins apparently were not present with greater frequency in the sickle cell positive group.

TABLE VI
SICKLE CELL TESTS AND COLD AGGLUTININ TITERS
IN PRE-EXPOSURE SUBJECTS

		SICKLE CELL NEGATIVE	SICKLE CELL POSITIVE	COMBINED
TOTAL		187	22	209
POSITIVE AGGL.		127 (68.0%)	18 (81.8%)	145 (69.4%)
NEGATIVE AGGL.		60 (32.0%)	4 (18.2%)	64 (30.6%)
TITERS	0	60	4	64
	1:2	44	6	50
	1:4	60	9	69
	1:8	10		10
	1:16	3	1	4
	1:32	8	2	10
	1:64	2		2

Table VII summarizes the results in 123 post-exposure cases. Again positive titers of cold agglutinins were not present with any greater frequency in the sickle cell positive group. The number of cases with positive agglutination may be significantly lower than the pre-exposure group ($p = 0.0145$); A pre-exposure figure of 69.4% positive (Table VI) is compared with a post-exposure figure of 56.1% positive (Table VII) cold agglutinins.

TABLE VII

SICKLE CELL TESTS AND COLD AGGLUTININ TITERS
IN POST-EXPOSURE SUBJECTS

		SICKLE CELL NEGATIVE	SICKLE CELL POSITIVE	COMBINED
TOTAL		111	12	123
POSITIVE AGGL.		63 (55.9%)	7 (58.4%)	69 (56.1%)
NEGATIVE AGGL.		49 (44.1%)	5 (41.6%)	54 (43.9%)
TITERS	0	49	5	54
	1:2	32	5	37
	1:4	24	2	26
	1:8	4		4
	1:16	1		1
	1:32			
	1:64	1		1

III. DISCUSSION

It is quite apparent from the data presented that the presence of the tendency to sickle red cells is not a predisposing factor in the development of frostbite in Negro troops. No evidence has been presented in the literature to show that the sickle cell trait has any causative relation to frostbite or cold, or that sickling "crises" in patients with sickle cell anemia are precipitated by low temperatures. The data presented substantiate the fact that cold exposure does not increase the tendency to sickle, again giving credence to the fact that the sickle cell trait is not influenced by external environmental factors during life, but is rather a constantly present inherited trait, a heterozygous Mendelian dominant. In its homozygous form it predisposes to sickle cell anemia.

Sufficient data is presented to show little or no correlation between the tendency to sickle and the presence of any particular blood group agglutinogen. Both sickling and blood groups are independent genetically acquired characteristics. Though cold agglutinins may be an environmentally acquired rather than an inherited trait (9), little relationship to sickling was found in the Negroes studied.

Since there is abundant crowding of interlocked sickle cells within the capillaries (in the patient with sickle anemia) thus producing thromboses, "sludging", and intravascular clumping, it would be of interest to study the capillary bed of a Negro with the sickle cell trait when exposed to low temperatures.

IV. CONCLUSIONS

In Negroes the presence of the sickle cell trait does not appear to be significantly related to the causation of frostbite.

The appearance of the sickle cell trait apparently is not influenced by exposure to cold.

Sickling has no apparent relationship to blood group agglutinogens in Negroes.

Sickling has no apparent relationship to cold agglutinins in control subjects and in frostbite cases.

V. RECOMMENDATIONS

None.

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